Inorganic Chemistry
Article

Inorg. Chem. **2009**, *48*, 10625–10634 **10625**
DOI: 10.1021/ic901159r

Investigation of the Coordination Interactions of S-(Pyridin-2-ylmethyl)-L-Cysteine Ligands with $M(CO)_3^+$ (M = Re, ^{99m}Tc)

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Received June 16, 2009

Development of new ligands for *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) led the investigation with S-(pyridin-2-ylmethyl)-L-cysteine, **1**. The ligand **1** has potential to coordinate with the metal through three different tridentate modes: tripodal through cysteine (O,N,S) and two linear involving the S-pyridyl and cysteine (O,S,N_{Py}, N,S,N_{Py}). From the reaction with **1**, two species were observed in the ^{1}H NMR, where the primary product was the linear *fac*-Re(N,S,N_{Py}-**1**)$(CO)_3^+$, **2a**, complex. To identify the coordination mode of the minor product, functionalized analogues of **1** were prepared from S-(pyridin-2-ylmethyl)-Boc-L-cysteine-methyl ester, **3**, with orthogonal protecting groups on the C terminus (methyl ester) in S-(pyridin-2-ylmethyl)-L-cysteine methyl ester, **4**, or N terminus (Boc) in S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, that specifically directed the coordination mode of *fac*-$M(H_2O)_3(CO)_3^+$ to either N,S, N_{Py} or O,S,N_{Py}, respectively. Two diastereomers [*fac*-$Re(CO)_3$(N,S,N_{Py}-**4**)]$^+$, **5a** and **5b**, were observed and independently characterized by X-ray structure analysis and NMR in high yield with **4**. Surprisingly, the O,S,N_{Py} Re complex with ligand **6** was not observed and simplified versions, 3-(pyridin-2-ylmethylthio) propanoic acid, **7**, and 2-(pyridin-2-ylmethylthio)acetic acid, **8**, were investigated. Ligand **7** did not yield the desired linear tridentate O,S,N_{Py} product. However, the shorter ligand **8** formed *fac*-$Re(CO)_3$(O,S,N_{Py}-**8**), **9**, in high yield. ^{99m}Tc labeling studies were conducted and yielded similar results to the rhenium complex and effective (>99%) at 10^{-5} M ligand concentration.

Introduction

In recent years, imaging and radio-therapeutic applications of the second and third row congeners, ^{99m}Tc (γ, 140 keV, $t_{1/2}$ = 6.0 h) and $^{186/188}$Re (β^-_{max} (1.1 MeV), $t_{1/2}$ = 3.7 d; β^-_{max} (2.1 MeV), $t_{1/2}$ = 17 h) has significantly increased in nuclear medicine.[1−3] The water-soluble organometallic *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) complex has yielded considerable interest because of the compact stable nature of the tricarbonyl core.[4−11] Several general coordination strategies (i.e., monodentate, "2 + 1", tridentate ligands) have been investigated with *fac*-$M(OH_2)_3(CO)_3^+$.[12−17] Tridentate ligands have typically offered higher chemical and biological stability that are further subdivided into two general classes: linear[18−22] and

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Published on Web 10/20/2009

tripodal.[20−29] Both classes utilize similar design strategies in donors atoms (i.e., N, O, P, S) and coordination ring size (5 or 6) to generate comparable complexes, albeit different ligand conformations.

Investigation of new bifunctional chelates (BFC) to effectively coordinate $M(CO)_3^+$ while linking a biological targeting molecule (i.e., receptor, transporter recognition) continues to be an active area of research. New methods are needed for radiolabeling for large peptides prepared by recombinant techniques rather than protecting group based solid phase synthesis. His-tag labeled peptides have provided initial success with $M(CO)_3^+$ by coordination through multiple imidazole donors with reasonable stability. However, the exact orientation of the metal center within His-tag remains undefined.[30] Alternative labeling strategies are needed to improve the specific $M(CO)_3^+$ coordination, while maintaining mild synthetic and minimal purification requirements found with His-tag.

The reactive nature of thiol in cysteine provides an excellent synthetic handle for conversion of the terminal amino acid into a tridentate ligand capable of coordinating the $M(CO)_3^+$ in a specific mode, while maintaining its association to a peptide. Several analogous linear tridentate and tripodal ligands utilizing thiols and thioethers for complexing $M(CO)_3^+$ provide a basis for determining a S-pendent donor.[26,31−34] Pyridine was selected for functionalizing of the cysteine moiety as it provides an excellent soft donor for the $M(CO)_3$ core and has been demonstrated as an effective ligand as reported with other metals.[35−38] The model ligand S-(pyridin-2-ylmethyl)-L-cysteine, **1**, was prepared and investigated to probe the ligand interactions with $M(CO)_3^+$. Because of the number of available donors (four) and coordination sites (three), ligand **1** has the potential to form three different tridentate coordination complexes with $M(CO)_3^+$, one tripodal (N,S,O of cysteine) and two linear

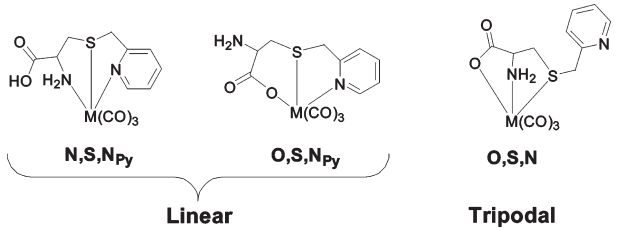

Figure 1. Three possible tridentate coordination compounds illustrating two types of coordination styles (Linear, Tripodal) that could be formed in the reaction of **1** with $M(OH_2)_3(CO)_3^+$ M = Re, ^{99m}Tc.

(N,S,N$_{Py}$ or O,S,N$_{Py}$) (Figure 1). Tripodal thioether S-functionalized cysteine complexes have been observed with non coordinating extensions (i.e., methyl, propyl, benzyl).[26,27] However, the addition of a S-pendent donor to cysteine has led to discussion of the types of complexes (tripodal vs linear) reported in the literature with $M(CO)_3^+$ because of the prochiral nature of the thioether. Two S functionalized aromatic thioether amino acid variations (S-(2-2′-pyridyl)ethyl-D,L-homocystiene (tripodal N,S,O) and 1,2,3 triazole cysteine (linear N,S,N$_{Tri}$ and tripodal N, S,O) reported in the literature have differing results with *fac*-$Re(CO)_3^+$.[29,32] Although these compounds are similar in nature, subtle difference in the donor strength of the S pendent ligand (pyridine vs triazole) and coordination ring size may have attributed to the observed differences in the isolated complexes.

In light of the ambiguity in the literature, we investigated ligand **1** for potential complex formation with *fac*-$M(CO)_3^+$ (M = Re, ^{99m}Tc). Different than previously investigated ligand systems, **1** is a composite ligand that features the strengths of both ligands, a smaller chelate ring size (cysteine vs homocysteine), and improved donor strength (pyridine vs triazole). The studies presented here provide a convenient aqueous synthetic route for preparing pyridine functionalized cysteine ligands and a fundamental evaluation of the coordination chemistry to determine the most effective coordination mode. **1** also provides important insights into evaluating the coordination of *fac*-$M(CO)_3^+$ in the presence of multiple donors. Protected ligand variations at the C and N terminus of **1** were also prepared to specifically control the coordination mode of the ligand (N,S,N$_{Py}$ or O,S,N$_{Py}$) with *fac*-$M(CO)_3^+$ (M = Re, ^{99m}Tc). These ligands provide essential controls to determine the effectiveness of the linear systems with $M(CO)_3$ and to simulate conditions found with peptide analogues prior to implementation in a biological targeting molecule.

Experimental Section

All reagents and organic solvents of reagent grade or better were used as purchased from Aldrich, Acros, or Fluka without further purification. Rhenium starting materials $Re(CO)_5SO_3CF_3$, and *fac*-$[Re(CO)_3(H_2O)_3][SO_3CF_3]$, were prepared by literature methods from $Re_2(CO)_{10}$ purchased from Strem.[12,26] Elemental analyses were performed by Quantitative Technologies, Inc., NJ. Analytical identification of compounds were conducted on a Perkin-Elmer Series 200 High Pressure Liquid Chromatograph (HPLC) equipped with a UV/vis Series 200 detector, a Radiomatic 610TR detector, and a 30 cm Agilent ZORBAX SB-C18 5 μm particle column using a reverse phase gradient system beginning with 0.1% trifluoroacetic acid (TFA) aqueous eluent gradually shifting to methanol was utilized: 0−3.0 min (100%

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TFA), 3.0−9.0 min (75% TFA, 25% MeOH), 9.0−20.0 min (25% to 100% MeOH linear gradient), 20.0−25.0 min (100% MeOH) at a flow rate of 1.0 mL/min. Semipreparatory separations were carried out with a Hitachi D-7000 series HPLC system equipped with a UV L-7400 detector on a 21.2 × 250 mm SB-C18 7 μm particle Agilent ZORBAX column at 10.0 mL/min under a similar gradient conditions as the analytic system. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz spectrometer, and chemical shifts were referenced to internal sodium 3-(trimethylsilyl)propionate-d_4 (TSP, 0.00 ppm) in D_2O or the residual solvent signal in organic solvents. Mass measurements were performed as Q3 scans on an Applied Biosystems AP14000 triple quadrupole.

S-(pyridin-2-ylmethyl)-L-cysteine, 1. 2-(Bromomethyl)pyridine hydrobromide (0.51 g, 2.0 mmol) and L-cysteine (0.32 g, 2.6 mmol) were added as a solid to 20 mL of water and adjusted to pH 8 by $NaHCO_3$ (1 M) followed by the addition of methanol (10 mL). The solution was stirred overnight at room temperature. The product was purified and isolated by preparatory HPLC as a colorless semisolid (0.62 g, 69%). ^{1}H NMR [δ(ppm), D_2O], 8.72 (d, 1H), 8.54 (dd, 1H), 8.06 (d, 1H), 7.94 (m, 1H), 4.20 (s, 2H), 4.12 (dd, 1H), 3.10 (dd, 1H), 3.02 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 171.4, 152.4, 147.5, 141.9, 127.9, 126.1, 52.8, 32.4, 31.5. MS [$(M+H)^+$], 213.

[*fac*-Re(CO)$_3$(N,S,N$_{Py}$-1)][CF$_3$SO$_3$], 2a and 2b. To a solution of **1** (0.17 g, 0.5 mmol) in 5 mL of deionized H_2O was added *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution (pH = 2) was heated at 70 °C for 2 h. $NaHCO_3$ was added periodically to adjust the solution to pH = 2. After heating, HPLC analysis showed a single peak (Rt = 17.54 min). After cooling, the mixture was concentrated and desalted on a Sephadex G-15 column (eluted with Deionized water) to give a white solid (0.27 g, 90%). ^{1}H NMR of the crude product showed the presence of two isomers indicated as **2a** (major isomer) and **2b** (minor isomer). Anal. Calcd for $C_{14}H_{12}F_3N_2O_7ReS$: C, 28.24; H, 2.03; N, 4.70. Found: C, 27.90; H, 1.94; N, 4.57.

2a. Pure crystals of major isomer **2a** were obtained by fractional recrystallization of the purified products (**2a** and **2b**) from deionized water. ^{1}H NMR of the isolated crystals indicated they were a single isomer corresponding to major species. X-ray quality crystals of **2a** were obtained by slow evaporation of the MeOH/H_2O solution. Anal. Calcd for $C_{14}H_{12}F_3N_2O_7ReS$: C, 28.24; H, 2.03; N, 4.70. Found: C, 27.76; H, 1.72; N, 4.55. ^{1}H NMR [δ(ppm), D_2O], 9.05 (d, 1H), 8.04 (dd, 1H), 7.75 (d, 1H), 7.46 (dd, 1H), 6.00 (d, 1H), 4.98 (d, J = 17.4 Hz, 1H), 4.58 (d, J = 17.4 Hz, 1H), 4.06 (dd, 1H), 3.82 (m, 2H), 3.59 (dd, 1H), 2.53 (dd, 1H). [δ(ppm),CD_3COCD_3], 9.19 (d, 1H), 8.14 (dd, 1H), 7.94 (d, 1H), 7.59 (dd, 1H), 6.21 (d, 1H), 5.30 (d, J = 17.4 Hz, 1H), 4.77 (d, J = 17.4 Hz, 1H), 4.24 (dd, 1H), 3.83 (m, 2H), 2.66 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 177.9, 161.8, 158.2, 143.5, 128.6, 128.2, 65.4, 48.2, 40.0. MS [M^+], 483, 481.

S-(pyridin-2-ylmethyl)-Boc-L-cysteine Methyl Ester, 3. To a solution of 2-(bromomethyl) pyridine hydrobromide (0.34 g, 1.3 mmol) in acetonitrile (10 mL) was added triethylamine (0.36 mL, 2.6 mmol), whereupon the solution turned pink. Then a solution of *N*-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (0.29 g, 1.2 mmol) in acetonitrile (5 mL) was added. The solution was stirred overnight at room temperature under N_2. The solution was concentrated to dryness and was purified by silica gel chromatography. The crude material was loaded as a dry solid onto a column conditioned with a combination of ethyl acetate: hexanes (1:6) that was gradually shifted to ethyl acetate: hexanes (1:3) to elute the product. Upon evaporation, the product was collected as colorless oil. (0.28 g, 71%) ^{1}H NMR [δ(ppm), CD_3COCD_3], 8.65 (d, 1H), 8.01 (dd, 1H), 7.65 (d, 1H), 7.49 (dd, 1H), 6.62 (b, 1H), 3.98 (dd, 2H), 3.68 (s, 3H), 4.42 (m, 1H), 3.00 (dd, 1H), 2.89 (dd, 1H), 1.48 (s, 9H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 172.2, 158.5, 156.3, 147.9, 140.3, 125.2, 123.9, 79.5, 54.5, 52.4, 36.8, 34.0, 28.5. MS [M^+], 326.

S-(pyridin-2-ylmethyl)-L-cysteine Methyl Ester, 4. To a solution of **3** (0.15 g, 0.38 mmol) in methylene chloride (2.7 mL) was added trifluoroacetic acid (0.3 mL) [1:4 TFA/CH_2Cl_2]. The solution was stirred overnight at room temperature, then evaporated to dryness. The product was isolated as a colorless oil (0.14 g, 95%). ^{1}H NMR [δ(ppm), D_2O], 8.73 (m, 1H), 8.54 (td, 1H), 8.02 (d, 1H), 7.96 (m, 1H), 4.40 (dd, 1H), 4.21 (s, 2H), 3.81 (s, 3H), 3.19 (dd, 1H), 3.06 (dd, 1H). ^{13}C NMR [δ(ppm), D_2O], 168.8, 152.2, 147.5, 142.0, 127.7, 126.1, 54.0, 52.1, 32.4, 30.9. MS [$(M+H)^+$], 227.

[*fac*-Re(CO)$_3$(N,S,N$_{Py}$-4)][CF$_3$CO$_2$], 5a and 5b. To a solution of **4** (0.17 g, 0.5 mmol) in deionized H_2O (5 mL) was added *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution (pH 2) was heated at 70 °C for 2 h. $NaHCO_3$ was added periodically to adjust pH back to 2. Analytical HPLC analysis of the reaction mixture showed two peaks with retention times (Rt) of 17.50 and 18.26 min. After cooling, the mixture was desalted by preparative HPLC, and the collected product was evaporated to dryness (0.29 g, 89%). ^{1}H NMR of the solid showed the presence of two isomers. Anal. Calcd for $C_{13}H_{14}N_2O_5ReS\cdot 2/3CF_3CO_2\cdot 1/3CF_3SO_3\cdot 1/3CF_3CO_2H$: C, 27.88; H, 2.17; N, 4.24. Found: C, 28.06; H, 1.75; N, 4.14. Two diastereomers (**5a** and **5b**) were separated and isolated by successive preparative HPLC purification.

5a. (Rt = 17.50 min). ^{1}H NMR [δ(ppm),CD_3COCD_3], 9.21 (d, 1H), 8.20 (td, 1H), 8.00 (d, 1H), 7.64 (m, 1H), 6.24 (d, 1H), 5.38 (d, J = 17.7 Hz, 1H), 4.80 (d, J = 17.7 Hz, 1H), 4.36 (dd, 1H), 4.07 (m, 2H), 3.63 (s, 3H), 2.74 (dd, 1H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 169.8, 159.7, 156.2, 140.9, 126.3, 125.9, 61.4, 52.6, 45.9, 35.9. MS [M^+], 497, 495. Crystals suitable for X-ray crystallography were obtained by slow evaporation of aqueous solution of **5a**.

5b. (Rt = 18.26 min). ^{1}H NMR [δ(ppm),CD_3COCD_3], 9.15 (d, 1H), 8.18 (t, 1H), 7.98 (d, 1H), 7.60 (t, 1H), 6.05 (d, 1H), 5.95 (dd, 1H), 5.38 (d, 1H), 4.92 (dd, 1H), 3.54 (s, 3H), 3.50 (m, 3H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 169.9, 160.6, 156.2, 140.7, 125.9, 125.0, 57.9, 52.6, 45.4, 37.5. MS [M^+], 497, 495. Crystals suitable for X-ray crystallography were obtained by diffusion of methyl *tert*-butyl ether into methanolic solution of **5b**.

S-(pyridin-2-ylmethyl)-Boc-L-cysteine, 6. To a solution of **3** (0.72 g, 2.2 mmol) in 15 mL of methanol was added 2.5 mL of 1 M NaOH and stirred at room temperature for 3 h. The solution was then neutralized with 2.5 mL of 1 M HCl and concentrated to ~5 mL by rotary evaporation then placed in the refrigerator overnight. The product was isolated as a colorless solid. (0.45 g, 65%). ^{1}H NMR [δ(ppm), CD_3COCD_3], 8.51 (d, 1H), 7.77 (dd, 1H), 7.46 (d, 1H), 7.26 (dd, 1H), 4.44 (m, 1H), 3.93 (dd, 2H), 3.05 (dd, 1H), 2.93 (dd, 1H), 1.42, (s, 9H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 171.9, 159.0, 155.6, 149.1, 137.2, 123.4, 122.3, 78.7, 53.6, 37.7, 33.5, 27.9. MS [M^+], 312.

3-(Pyridin-2-ylmethylthio)propanoic acid, 7. To a solution of 2-picolyl chloride hydrochloride (1.64 g, 10 mmol) and methyl 3-mercapto propanoicate (1.2 mL, 10.8 mmol) in acetonitrile (40 mL) was added cesium carbonate (7.15 g, 22 mmol). The mixture was stirred overnight at room temperature and filtered. The filtrate was evaporated to dryness to yield the crude methyl ester product. The methyl ester intermediate was redissolved in 10 mL of MeOH, followed by 10 mL of 1 M NaOH solution. The solution was stirred for 6 h then neutralized with 12 mL of 1.0 M HCl solution. The product was purified by preparative HPLC yielding a white solid (1.63 g, 83%). ^{1}H NMR [δ(ppm), D_2O], 8.52 (d, 1H), 8.36 (dd, 1H), 7.88 (d, 1H), 7.78 (dd, 1H), 4.01 (s, 2H), 2.60 (t, 2H), 2.47 (t, 2H). ^{13}C NMR [δ(ppm), CD_3COCD_3], 172.8, 160.2, 150.0, 145.3, 132.1.2, 129.8, 55.5, 38.7, 35.6. MS [$(M-H)^-$], 196.

2-(Pyridin-2-ylmethylthio)acetic acid, 8. 2-Picolyl chloride hydrochloride (1.64 g, 10 mmol) and mercaptoacetic acid (0.77 mL, 11 mmol) in H_2O (40 mL) was added dropwise to NaOH (16 mL, 1 M) in 1 h. The mixture was stirred overnight,

Table 1. Crystallographic Data and Structure Refinement Parameters for compound **2a**, **5a**, **5b**, and **9**

	2a	5a	5b	9
formula	$C_{14}H_{12}F_3N_2O_7ReS$	$C_{14}H_{14}F_3N_2O_8ReS_2$	$C_{15}H_{14}F_3N_2O_7ReS$	$C_{11}H_8NO_5ReS$
formula weight	595.52	645.59	609.54	452.44
space group	P2(1)2(1)2(1)	P2(1)/n	P2(1)2(1)2(1)	P-1
a (Å)	8.2755(3)	8.6579(5)	10.7633(12)	7.2461(8)
b (Å)	10.7053(4)	17.2829(10)	16.2152(17)	9.3299(10)
c (Å)	20.7099(7)	13.1663(8)	33.494(4)	10.3539(11)
α (deg)				101.925(5)
β (deg)		96.3680(10)		106.904(5)
γ (deg)				101.832(5)
V (Å^3)	1834.73(11)	1958.02(2)	5845.68(11)	628.45(12)
Z	4	4	12	4
T (K)	90(2)	90(2)	190(1)	190(1)
D_{calcd} (Mg/m^3)	2.156	2.190	2.078	2.391
μ (mm^{-1})	6.807	6.494	6.412	9.848
GOF	1.034	1.038	1.06	1.113
R_1 [$I > 2\sigma(I)$][a]	0.0162	0.0192	0.0246	0.0213
wR_2 [$I > 2\sigma(I)$][a]	0.0336	0.0436	0.0309	0.0549

[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$; $wR_2 = \{\sum w(F_o^2 - F_c^2)^2/\sum w(F_o^2)^2\}^{1/2}$.

neutralized to pH 2 with HCl, and concentrated to 20 mL. A dark solid was formed, collected, and decolored by charcoal in EtOH-H_2O solution. Concentration of the colorless filtrate to ~10 mL yielded the product as a solid, which was washed sparingly with water and collected. (0.65 g, 36%). ^{1}H NMR [δ(ppm), D_2O/NaOD], 8.27 (d, 1H), 7.64 (dd, 1H), 7.18 (d, 1H), 7.15 (dd, 1H), 3.66 (s, 2H), 2.93 (s, 2H). ^{13}C NMR [δ(ppm), D_2O/NaOD], 180.2, 159.6, 151.2, 141.5, 127.1, 125.8, 39.6, 38.9. MS [M$^+$], 183.

***fac*-Re(CO)$_3$(O,S,N$_{Py}$-8), 9**. To a solution of **8** (0.10 g, 0.55 mmol) in MeOH (5 mL) was added aqueous solution of *fac*-[$Re(CO)_3(H_2O)_3$][SO_3CF_3] (5 mL, 0.1 M). The solution was refluxed for 2 h and HPLC analysis showed only a peak with Rt of 19.14 min. The mixture was concentrated to 5 mL producing a precipitate (0.20 g, 90%). Suitable X-ray quality single crystals were prepared by recrystallizing **9** in CH_2Cl_2/acetone to yield colorless plates. Anal. Calcd for $C_{11}H_8N_2O_5ReS \cdot 0.2CH_2Cl_2$: C, 28.59; H, 1.78; N, 2.98. Found: C, 28.17; H, 1.42; N, 2.98. ^{1}H NMR [δ(ppm), CD_3COCD_3], 9.05 (d, 1H), 8.17 (dd, 1H), 7.91 (d, 1H), 7.59 (dd, 1H), 5.12 (d, J = 17.4 Hz, 1H), 4.74 (d, J = 17.4, Hz 1H), 3.58 (d, J = 17.7 Hz, 1H), 3.20 (d, J = 17.7 Hz, 1H). ^{13}C NMR [δ(ppm), d_6-DMSO], 179.3, 160.2, 154.9, 141.2, 126.3, 125.9, 46.0, 33.4. MS [(M − H)$^-$], 452.

X-ray Experimental. Crystals of compounds **2a**, **5a**, **5b**, and **9** were removed from the flask and covered with a layer of hydrocarbon oil. A suitable crystal was selected, attached to a glass fiber, and placed in the low-temperature nitrogen stream.[39] Data (**2a** and **5a**) were collected at low temperatures using a Bruker/Siemens SMART APEX instrument (Mo Kα radiation, λ = 0.71073 Å) equipped with a Cryocool NeverIce low temperature device. Data for **2a** and **5a** was measured using omega scans of 0.3° per frame for various exposures, and a full sphere of data was collected in each case. A total of 2400 frames were collected with final resolutions of 0.77 Å. Cell parameters were retrieved using SMART[40] software and refined using SAINTPlus[41] on all observed reflections. Data reduction and correction for Lp and decay were performed using the SAINTPlus software. Absorption corrections were applied using SADABS.[42] Data for **5b** and **9** were collected at low temperature on a Nonius Kappa CCD (Mo Kα radiation, λ = 0.71073 Å) using phi and omega scans. A total of 893 and 522 frames were collected for **5b** and **9** respectively. The unit cells were refined with 25460 reflections for **5b** and 2994 reflections for **9**. The diffraction intensities for **5b** and **9** were collected to a 0.76 Å resolution. Absorption corrections were performed using HKL Scalepack. Structures **2a** and **5a** were solved by direct methods and refined by the least squares method on F^2 using the SHELXTL program[43] package. Structures **5b** and **9** were solved by either the heavy-atom (**5b**) or direct (**9**) method using SHELXS-97.[44] The least squares refinements performed were conducted with SHELXL-97.[44] All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were in all cases refined in geometrically constrained riding positions. No decomposition of crystals was observed during the data collection process. Details of the data collection and refinement are given in Table 1. Further experimental details are provided in the Supporting Information.

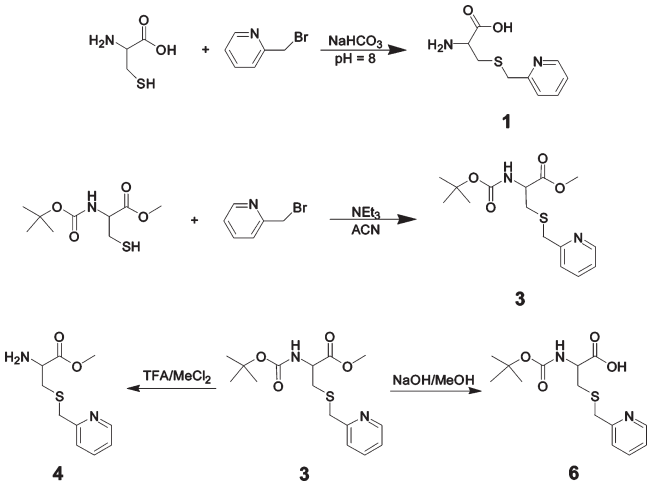
General ^{99m}Tc(H$_2$O)$_3$(CO)$_3^+$ Radiolabeling Procedure. The ligand (100 μL, 10^{-4} or 10^{-5} M) and phosphate buffer (800 μL, 0.1 M) at pH 5.0 or 7.4 was added to a sealable labeling vial (5.0 mL). The vial was sealed and degassed with nitrogen for ~10 min. The $^{99m}Tc(H_2O)_3(CO)_3^+$ precursor solution (100 μL) prepared according to the Isolink kit from Tyco specifications was added to the degassed vial, and the vial heated for 1 h at 90 °C. The reaction mixture was carefully allowed to cool on an ice bath prior to injection and analysis by radio-HPLC.

Results and Discussion

Several variations of S-(pyridin-2-ylmethyl)-L-cysteine ligands were synthesized in a straightforward manner (Scheme 1). The general strategy involved a similar approach to other cysteine compounds reported in the literature.[35,36,45] A protected cysteine at the C and N terminus (Boc-Cys-OMe) was reacted with 2-(bromomethyl) pyridine in acetonitrile in the presence of a base to yield the product S-(pyridin-2-ylmethyl)-Boc-L-cysteine methyl ester, **3**. The orthogonal protecting groups could be selectively removed by either acid (trifluoroacetic acid/methylenechloride) or basic (NaOH/methanol) conditions to yield S-(pyridin-2-ylmethyl)-L-cysteine methyl ester, **4**, and S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, respectively. Both groups could be subsequently deprotected to generate the unprotected version S-(pyridin-2-ylmethyl)-L-cysteine, **1**, in three steps.

(39) Hope, H. *Prog. Inorg. Chem.* **1994**, *41*, 1–19.
(40) *SMART*; Bruker AXS: Madison, WI, 2001.
(41) *SAINTPlus*; Bruker AXS: Madison, WI, 2003/2004.
(42) *SADABS*; Bruker AXS: Madison, WI, 2004.
(43) *SHELXTL*; Bruker AXS: Madison, WI, 2004.
(44) Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *64*, 112–122.
(45) Ijuin, R.; Umezawa, N.; Higuchi, T. *Bioorg. Med. Chem.* **2006**, *14*, 3563–3570.

Scheme 1. Synthesis of S-Functionalized S-(pyridin-2-ylmethyl)-L-cysteine Ligands



To minimize multistep synthesis for application with peptides, an alternative strategy to direct alkylation of the thiol of cysteine without the use of protecting groups was investigated. Literature methods for aqueous alkylation of the thiol in cysteine utilize strongly basic conditions (i.e., 1 M NaOH) and high temperature (100 °C), which may present problems for pH sensitive peptides because of racemization of the amino acids and structural deformations. To address this issue, cysteine was utilized as a model for peptides to investigate mild aqueous alkylation conditions of thiols. It was found that S-(pyridin-2-ylmethyl)-L-cysteine, **1**, could be prepared by directly reacting cysteine without the use of protecting groups with 2-(bromomethyl) pyridine at pH ~8 in a bicarbonate buffer at room temperature overnight. Only the S alkylated product **1** was observed in the reaction mixture identified by ^{1}H NMR and purified by preparatory HPLC in good yield (69%). The new thiol alkylation conditions provide a facile strategy for generating S functionalized cysteine ligands for peptide applications, while minimizing possible side reactions caused by high pH and heating.

The S-(pyridin-2-ylmethyl)-L-cysteine ligands were reacted with *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc) to elucidate the possible coordination modes, tripodal (N,S,O of cysteine) or linear (N,S,N_{Py} or O,S,N_{Py}), that would be observed by the ligand.

Each of the three ligands (**1**, **4**, and **6**) representing the different types of $M(CO)_3$ complexes was examined to provide essential information on how to best incorporate them into targeting molecules. Ligand **1** has the potential to form all three types of complexes. Whereas, ligands **4** and **6** reduce the ambiguity to a single coordination mode through selective removal of only one of the protecting groups yielding linear tridentate ligands and $M(CO)_3$ complexes (N,S,N_{Py} or O,S,N_{Py}), respectively. Together these ligands provided important insights in the specific interactions of $M(CO)_3$ with S-(pyridin-2-ylmethyl)-L-cysteine ligands.

In the reaction of **1** with *fac*-$[Re(OH_2)_3(CO)_3]^+$, several factors such as overall complex charge, neutral (tripodal and linear O,S,N) versus cationic (linear N,S,N), chelate ring size, donor ligands, and conformation (linear vs tripodal) were anticipated to affect the coordination modes observed with $M(CO)_3$ and potentially yield several species in the reaction mixture. HPLC analysis throughout the course of the reaction yielded a single peak at 17.5 min (Figure 2). This observation did not preclude the possibility of multiple coordination modes present in the sample, yet insufficient separation of the species under the column conditions. ^{1}H NMR was conducted on the isolated peak and revealed two distinct sets of signals that indicated two products in a 6:1 ratio. Both products exhibited similar H_a-H_b splitting of the methylene protons (S-CH_2-Pyr). The doublets were slightly shifted (4.58, 4.98 ppm) and (4.8, 5.3 ppm), but had identical coupling constants (J = 17.4) for each complex. Whereas, H_a-H_b splitting of the peak of the similar methylene protons (S-CH_2-Ph) in the tripodal *fac*-$Re(CO)_3$(S-benzyl-cystiene) complex was not previously observed.[27] This suggested pyridine was involved in metal coordination in both species and eliminated tripodal as a possible coordination mode present in the sample. However, noticeable shifts in ^{1}H NMR of the cysteine protons (CH−CH_2−S) between the major and the minor species were noted and suggested distinctly different compounds.

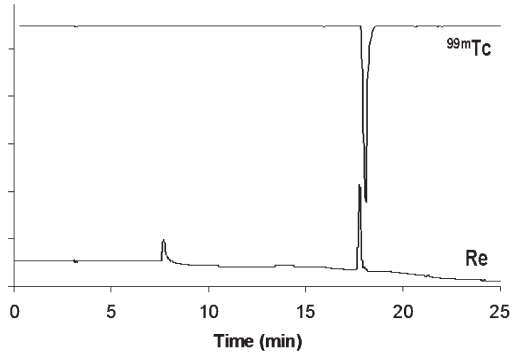
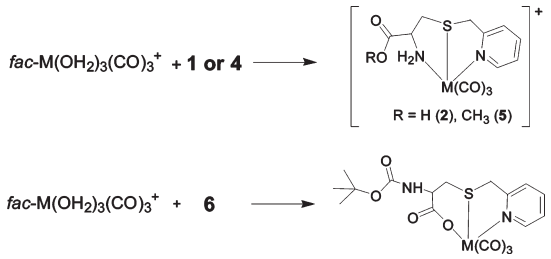


Figure 2. HPLC chromatograms of the reaction of **1** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom), and ^{99m}Tc (NaI, top) to yield *fac*-$[M(CO)_3(N,S,N_{Py}$-**1**$)]^+$, **2a** and **2b**.

Scheme 2. Reaction of S-(pyridin-2-ylmethyl)-L-cysteine Ligands with *fac*-$M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc)



Attempts to separate the two products by chromatographic methods were unsuccessful. However, fractional recrystallization of the crude material led to the isolation of the major species of the reaction [*fac*-$Re(CO)_3(N,S,N_{Py}$-**1**$)$]-$[CF_3CO_2]$, **2a**, (Scheme 2). Single crystal X-ray diffraction analysis of the isolated crystals definitively illustrated the N, S,N_{Py} orientation of **1** on the metal center (Figure 3). Crystallographic parameters and selected bond angles and distances for **2a** are found in Tables 1 and 2 and are discussed in more detail later. ^{1}H NMR of the isolated crystals **2a** was correlated with the crude sample, where **2a** was observed as the major product (Figure 4). However, isolation of a pure sample of the second minor product from the mother liquor proved elusive as trace amounts of the major product **2a** present in the sample cocrystallized with the minor product **2b**.

In an effort to better understand the ligand interactions of **1**, identify the minor species of **2b**, and evaluate the feasibility of S-(pyridin-2-ylmethyl)-L-cysteine ligands for C or N terminal ligands in peptides, ligands **4** and **6** were investigated with *fac*-$Re(OH_2)_3(CO)_3^+$. The protecting group (methyl ester or boc) on **4** and **6** would specifically limit the formation of a single coordination mode of $M(CO)_3$ to either N,S,N_{Py}, or O,S,N_{Py}, respectively (Scheme 2).

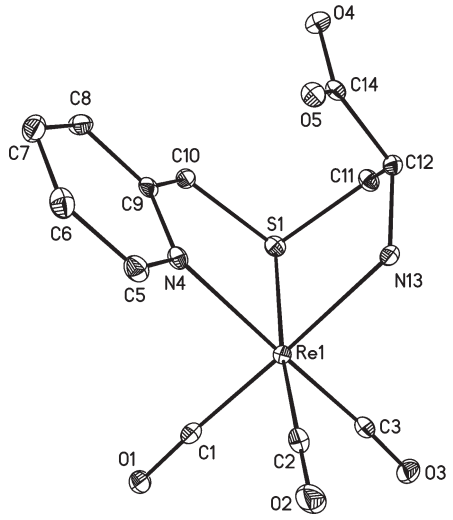


Figure 3. Molecular structure of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{1})]^+$, **2a**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

Table 2. Selected Bond Distances (Å) and Angles (deg) for Compounds **2a**, **5a**, **5b**, and **9**

	2a	5a	5b	9
$Re-NH_2$	2.227(3)	2.219(2)	2.220(5)	
$Re-N_{Py}$	2.210(2)	2.205(2)	2.218(5)	2.203(3)
Re−S	2.4516(8)	2.4530(7)	2.4468(2)	2.4529(10)
Re−CO	1.924(3)	1.932(3)	1.928(7)	1.915(5)
	1.922(3)	1.922(3)	1.942(8)	1.942(4)
	1.918(3)	1.908(3)	1.943(7)	1.903(5)
Re−O				2.138(3)
$S-CH_2$	1.809(3)	1.830(3)	1.834(6)	1.818(4)
	1.822(3)	1.810(3)	1.811(6)	1.808(4)
$N_{Py}-Re-NH_2$	85.26(10)	84.85(9)	83.69(18)	
NH_2-Re-S	81.29(7)	81.27(6)	80.77(12)	
$N_{Py}-Re-S$	80.39(7)	80.52(6)	79.23(13)	79.50(9)
$N_{Py}-Re-O$				78.73(10)
O−Re−S				80.65(8)
CH_2-S-CH_2	102.67(14)	101.78(15)	101.2(3)	101.9(2)

The reaction of **4** with *fac*-$Re(OH_2)_3(CO)_3^+$ yielded similarities and differences to those observed with ligand **1**. Two peaks of near equal intensity at 17.5 and 18.3 min were observed in the reaction mixture by HPLC chromatogram (Figure 5). ^1H NMR spectra of the crude reaction mixture were nearly identical of those of the crude product of **2a** and **2b** except that the former exhibits two additional singlet signals for methyl esters. Each of these peaks was separated by preparatory HPLC and fully characterized. The purified product (17.5 min) was found to be the anticipated complex [*fac*-$Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})][CF_3CO_2]$, **5a**. X-ray analysis of the single crystals of **5a** had the same N,S,N_{Py} coordination and structural configuration as **2a** (Figure 6). ^1H NMR of sample **5a** yielded nearly identical splitting patterns and peaks as identified for **2a** with the addition of the singlet for the methyl ester at 3.63 ppm. The second product (18.3 min) isolated from prep HPLC was found to be the diastereomer [*fac*-$Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})][CF_3CO_2]$, **5b**. Single crystals suitable for X-ray analysis of **5b** confirmed the N,S, N_{Py} orientation of **4** and the intact methyl ester (Figure 7). ^1H NMR spectra of **5b** available in the Supporting Information except for a methyl ester singlet was found to correlate with the minor species **2b** identified in the reaction mixture with

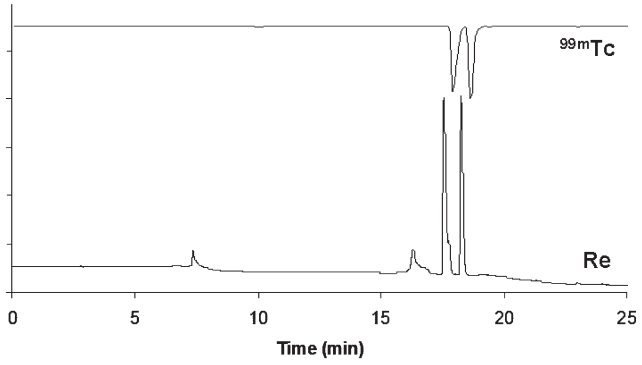


Figure 5. HPLC chromatograms of the reaction of **4** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom), and ^{99m}Tc (NaI, top) to yield *fac*-$[M(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})]^+$, **5a** (1st peak) and **5b** (2nd peak).

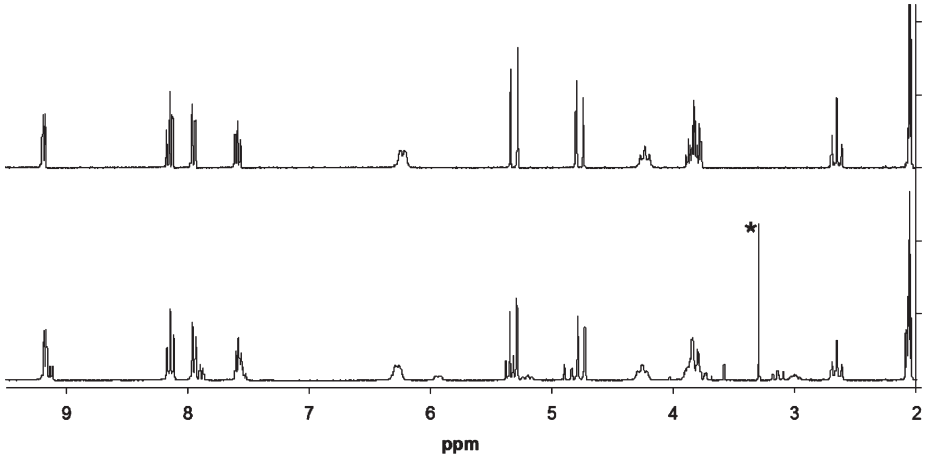


Figure 4. ^1H NMR spectra observed for the major species, **2a**, isolated as crystals (top) and unpurified reaction mixture (bottom) of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{1})]^+$, **2a** and **2b**.. *Residual methanol (3.3 ppm)

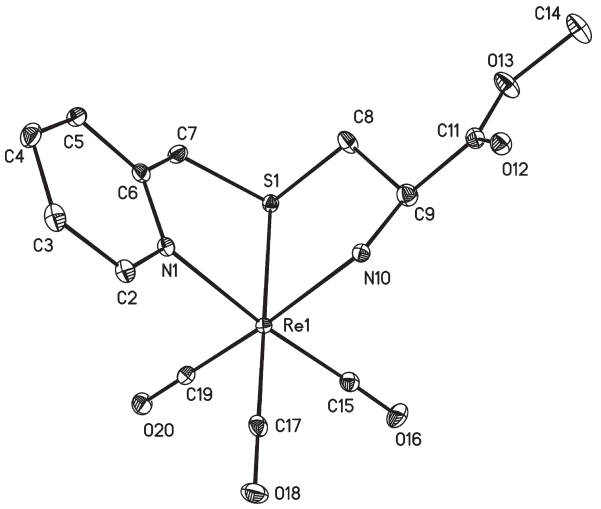


Figure 6. Molecular structure of *fac*-$[Re(CO)_3(N,S,N_{Py}\text{-}\mathbf{4})]^+$, **5a**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

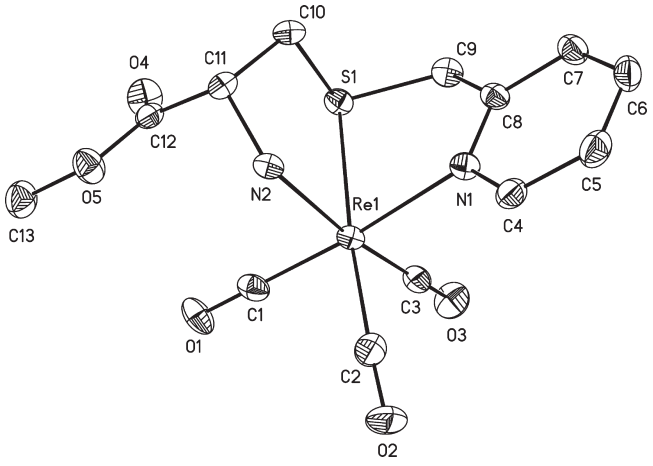


Figure 7. Molecular structure of *fac*-$[Re(CO)_3(NSN_{Py}\text{-}\mathbf{4})]^+$, **5b**. Thermal ellipsoids are shown at the 30% probability level. Hydrogen atoms omitted and only the Re cation is shown for clarity.

ligand **1**. A structural overlay of the two diastereomers (**5a**, **5b**) illustrated the differences in the absolute configurations of **4**, which is believed to have impacted the ^{1}H NMR spectra (Figure 8). In particular, the orientation of the methyl ester within the complex was found to be either pointing away from the molecule (**5a**) or parallel to Re-CO (**5b**), which shifted the H_α and H_β of cysteine into different magnetic environments. Interestingly to note, the reaction pH was critical to the purity and isolation of **5a** and **5b**. Complexation at neutral or slightly basic conditions yielded an additional peak in the chromatogram at 17.6 min. ^{1}H NMR of this sample indicated hydrolysis of the methyl ester and the presence of the N,S,N_{Py} complexes (**2a**, **2b**) as well as (**5a**, **5b**). Conducting the complexation under acidic conditions avoided the issue of methyl ester hydrolysis and permitted the formation of only **5a** and **5b**.

To ensure the correct assessment of the N,S,N_{Py} linear diastereomeric coordination mode of **2b**, the linear S-(pyridin-2-ylmethyl)-Boc-L-cysteine, **6**, ligand that permitted only O,S,N_{Py} coordination of the $M(CO)_3^+$ complex was also investigated. The reaction of *fac*-$Re(OH_2)_3(CO)_3^+$ with **6** did not proceed as expected. A number of low yield

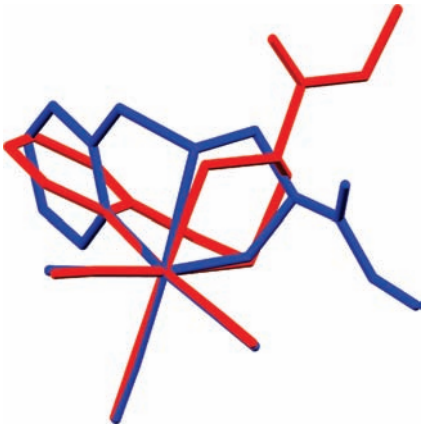
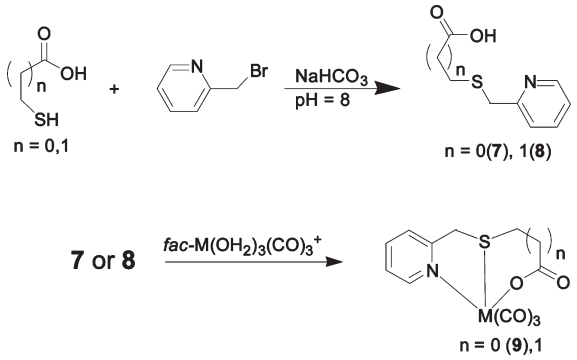


Figure 8. Structural overlay of the L-diasteromers **5a** (red) and **5b** (blue) illustrating ligand orientation of **4** in the complexes. Hydrogen atoms and atom labels omitted for clarity.

Scheme 3. Synthesis of O,S,N_{Py} Coordinating S-(pyridin-2-ylmethyl) Derived Ligands (**7**, **8**) and Subsequent Reactions with $M(OH_2)_3(CO)_3^+$ (M = Re, ^{99m}Tc)



species were observed by HPLC chromatogram with no single predominant species as in reactions with **1** or **4**. This result was unanticipated as the O,S,N_{Py} coordination of **6** would have generated five and six member coordination rings with the rhenium center, well within the expected realm of linear tridentate ligands for $Re(CO)_3^+$. Multiple attempts to isolate any compound from the reaction mixture proved unsuccessful. Additional synthetic approaches, such as adjusting pH (2−9), organic solvents (MeOH, $MeCl_2$), and other rhenium starting materials ($ReBr(CO)_5$, $[Re(CO)_5]^+$), did not yield the desired O,S,N_{Py} coordinated species either.

It was postulated that the steric bulk of the boc group and the proximity of the amine may be affecting the O,S,N_{Py} coordination of the ligand with the $Re(CO)_3^+$ core. Simplified variations eliminating steric bulk and chirality of cystiene, 3-(pyridin-2-ylmethylthio)propanoic acid, **7** and 2-(pyridin-2-ylmethylthio)acetic acid, **8**, were prepared by reacting the respective mercapto carboxylic acid with 2-picolyl chloride to generate the thioether pyridine methyl analogues (Scheme 3). Ligands **7** and **8** were reacted independently with *fac*-$Re(OH_2)_3(CO)_3^+$ to investigate O,S,N_{Py} complex formation (Scheme 3). The reaction of **7** with *fac*-$Re(OH_2)_3(CO)_3^+$ did not yield the expected O,S,N_{Py} complex under all the conditions examined (pH, solvent, and rhenium starting material). The results observed with **7** were similar to the cysteine analogue **6** indicating the steric effect of the boc amine group may not have impacted O,S,

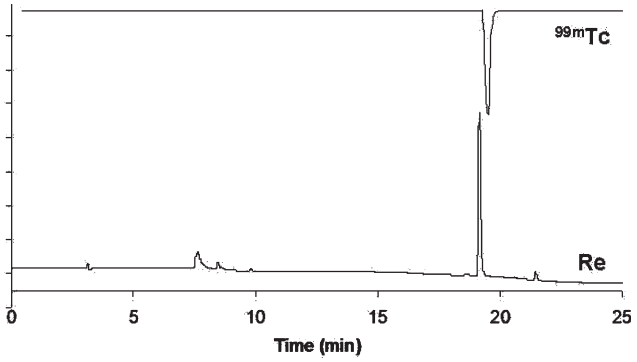


Figure 9. HPLC chromatograms of the reaction of **8** with $M(OH_2)_3(CO)_3^+$, Re (UV 220 nm, bottom) and ^{99m}Tc (NaI, top) to yield *fac*-[$M(CO)_3(N,S,N_{Py}$-**1**$)]^+$, **9**.

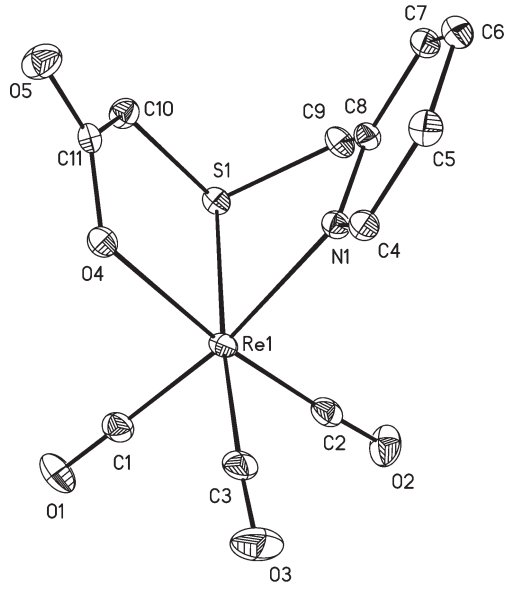


Figure 10. Molecular structure of *fac*-[$Re(CO)_3(O,S,N_{Py}$-**8**$)$], **9**. Thermal ellipsoids are shown at the 30% probability level and the hydrogen atoms were omitted for clarity.

N_{Py} complex formation and indirectly supporting the formation N,S,N_{Py} diastereomer **2b** from **1**.

Ligand **8** reduced the coordination ring size from six to five by removing one methylene group between the carboxylic acid and the sulfur. Unlike ligands **6** and **7**, the reaction of *fac*-$Re(OH_2)_3(CO)_3^+$ with **8** generated the expected product *fac*-$Re(CO)_3(O,S,N_{Py}$-**8**$)$, **9**, in near quantitative yield as a single product by HPLC at 19.1 min (Figure 9). Single crystals of **9** for X-ray diffraction analysis were obtained by slow evaporation of an acetone solution that is discussed later (Figure 10). The ^1H NMR spectrum of **9** shows the typical chemical shifts of the O,S,N_{Py} ligand coordinated by $Re(CO)_3$. H_a-H_b splitting of the methylene protons, SCH_2CO_2 and SCH_2Py, were observed as doublets (3.60, 3.20 ppm with $J = 17.7$ and 5.12, 4.75 ppm with $J = 17.4$), respectively. In particular, the chemical shifts and coupling constants of SCH_2Py and aromatic pyridine protons in **9** corresponded to those observed with the pyridine coordinated complexes, **2a**, **2b**, **5a**, and **5b**. The change in ring size from six in **7** to five in **8** directly impacted the successful isolation of the O,S,N_{Py} coordinated $Re(CO)_3^+$ complex.

X-ray Structure Analysis. Complexes **2a**, **5a**, **5b**, and **9** were characterized by single crystal X-ray diffraction analysis. The crystallographic experimental data, space group, and structure refinement parameters for the crystals are reported in Tables 1. Selected bond angles and distances for the complexes are found in Table 2. Complexes **2a**, **5a**, **5b**, and **9** were distorted octahedral complexes with the facial orientation of the carbonyl ligands nearly equidistant bond lengths (Re−C 1.90−1.94 Å) and bond angles (86.6−88.6°) indicative of $Re(CO)_3(L)$ complexes. In each of the complexes, the ligands (N,S,N_{Py} or $O,S,N_{Py,}$) were coordinated in a linear tridentate fashion around the metal center. Both types of complexes are composed of two slightly distorted five member coordination rings formed along the axis of ligand, which yielded several similarities in the structures. The elongated Re−S (∼2.45 Å) and C−S (1.81−1.83 Å) bonds significantly attributed to this perturbation compared to related Re−N (∼2.2 Å) and C−N or C−C (∼1.5 Å) bonds. The bond angles in the N,S,N_{Py} complexes (**2a**, **5a**, **5b**) between the N_{Py}−Re-S (79.2−80.5°) and N−Re−S (80.8−81.3°) and the bond distances (Re−N (∼2.22 Å), Re−S (∼2.45 Å), Re−N_{Py} (∼2.21 Å)) were generally consistent between each of the structures in terms of bonding of the ligand with the metal center.

Although the structures (**2a**, **5a**, **5b**) had similar bond angles and distances, the absolute configuration of the cysteine ligand yielded two distinct configurations on the metal center. The α-carbon on cysteine provided the impetus for diastereomer formation as it dictated the orientation of the uncoordinated carboxylic acid or methyl ester group. In the first diastereomer, the uncoordinated group (carboxylic acid, **2a** or methyl ester, **5a**) is oriented away from the $Re(CO)_3$ moiety and bent backward toward the coordinated ligand. This feature is more pronounced in **2a** than **5a** because of a hydrogen bond (3.114 Å) of the carbonyl (O5) with the pyridine ring (N4). Interestingly, **5a** crystallized in an achiral space group with the entantomeric pair equally observed in the structure, which mostly likely occurred because of racemization of ligand or complex.[38,46−48] In the second diastereomer **5b**, the methyl ester group of the ligand was orientated along the carbonyl bond of the $Re(CO)_3$ moiety and away from the coordinated ligand. Structural comparison of the ligand orientation of two diastereomers (**5a**, **5b**) is represented in the structural overlay of complexes (Figure 8).

Although ligand **8** has a carboxylate donor instead of an amine, the structure of the O,S,N_{Py} rhenium complex **9** was similar to the N,S,N_{Py} rhenium complexes in bond distances (Re−N_{Py} (2.20 Å), Re−S (2.45 Å)) and ligand conformation. The ligand in **9** appears to be more constrained with smaller coordination ring bond angles (O−Re−S (80.65 Å) and N_{Py}−Re-S (79.51 Å) and O−Re−N_{Py} (78.73 Å)) than the N,S,N_{Py} complexes. The observed differences are most likely due to a shorter Re−O bond (2.14 Å) and the sp^2 hybridization of the carboxylate, but are well within ranges for other thio/thioether complexes with $Re(CO)_3$.[19,26,32,34]

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The metal chelate ring size appears to be the critical factor for the formation of the Re complex because of the longer C−S bonds (~1.8 Å) compared to typical C−C or C−N bond (~1.5 Å) and Re−S (2.45 Å) distances to Re-NH_2 or ReN_{Py} (~2.2 Å). On the basis of the reported data, it is not as surprising that the ligand S-(2-(2′-pyridyl)ethyl)-D,L-homocystiene prefers a tripodal (N,S,O) coordination mode of homocysteine residue with Re-$(CO)_3$, even though pyridine is a potent donor.[32] The impact of the ethyl linker and longer covalent/coordination bonds of the thioether may have limited the participation of pyridine donor favoring the tripodal conformation. Also, the preferential formation of N,S,N_{Py} Re$(CO)_3$ diastereomers reported here over neutral complexes tripodal or linear (O,S,N_{Py}) modes highlights the importance of the donor strength of the pendent ligand. The previous report of S functionalized triazole cysteine Re$(CO)_3$ complexes suggested two different species, tripodal (N,S,O) and a linear (N,S,N_{Tri}), were formed in the absence of X-ray data.[29] Whereas, the S-(pyridin-2-ylmethyl)-L-cysteine Re$(CO)_3^+$ complexes clearly yielded N,S,N_{Py} diastereomers.

^{99m}Tc$(CO)_3^+$ Labeling Studies. To compare to the rhenium complexes, the ligands were also reacted with ^{99m}Tc$(OH_2)_3(CO)_3^+$ to determine radiolabeling yields at different ligand concentrations (10^{-5} and 10^{-6} M) and pHs (5.0 and 7.4) (Table 3). The reaction of **1** with ^{99m}Tc$(OH_2)_3(CO)_3^+$ yielded a single peak at 17.6 min of the product ^{99m}Tc(N,S,N_{Py}-**1**)$(CO)_3^+$, **2a** + **2b**, which correlated with the observed HPLC peak for the rhenium analogue (Figure 2). The labeling results were comparable at >99% at 10^{-5} M for both pH values. At 10^{-6} M, the formation of **2a** was significantly higher at pH 7.4 (86%) over pH 5.0 (44%). The lower yield at pH 5.0 is most likely due to protonation of the amine donor and electrostatic repulsion of the Tc core and the ligand. The reaction of **4** with ^{99m}Tc$(OH_2)_3(CO)_3^+$ yielded the expected products ^{99m}Tc(N,S,N_{Py}-**4**)$(CO)_3^+$, **5a** + **5b**, as two separate peaks of equal intensity (17.7 and 18.4 min) in the HPLC chromatogram in good yields at 10^{-5} and 10^{-6} M (Figure 5). The relative ratio of the two peaks observed at pH 5.0 correlated well with the rhenium complexes. Interestingly, at pH 7.4 the ratio of the two peaks shifted to favor the ~17.7 min peak (~60:40) at both concentrations. The ratio shift is most likely due to hydrolysis of the methyl ester in the ligand **4** as a faint shoulder of the peak on the front end of the 17.7 min peak indicative of the complex observed with ligand **1**. Prolonged heating of the sample at pH 7.4 confirmed hydrolysis as only a single peak at 17.6 min that corresponded to the results observed with ligand **1**.

The O,S,N_{Py} ligands (**6**, **7**, **8**) based on the cysteine backbone were investigated to determine reactivity with ^{99m}Tc$(OH_2)_3(CO)_3^+$. Even though we were unable to isolate the corresponding rhenium complexes, ligands (**6**, **7**) were examined and yielded the proposed compounds ^{99m}Tc(O,S,N_{Py}-**6**)$(CO)_3$ and ^{99m}Tc(O,S,N_{Py}-**7**)$(CO)_3$ in reasonable labeling yields at 10^{-5} M, but declined at 10^{-6} M. As anticipated by the observations of the N,S,N ligand, **4**, the complex of the boc protected cysteine ligand **6** yielded two peaks (17.6, 18.6 min) of unequal intensity (75:25) in the radiochromatogram, whereas ligand **7** had a single peak at 19.4 min with the ^{99m}Tc precursor. It was surprising based on the similar nature of the ligands, the labeling yields with ^{99m}Tc$(OH_2)_3(CO)_3^+$ for the cysteine analogue **6** were markedly superior than the linear analogue **7**. Even though labeling of these ligands was achieved, the exact speciation of the complexes is uncertain without structural conformation of the ^{99}Tc complexes. However, it is reasonable to propose a tridentate or bidentate (S,N_{Py}) complex with ^{99m}Tc-$(CO)_3^+$. The truncated O,S,N_{Py} ligand **8** was also reacted with ^{99m}Tc$(OH_2)_3(CO)_3^+$ to yield a single product ^{99m}Tc-(O,S,N_{Py}-**8**)$(CO)_3$, **9**, at 19.2 min that correlated with the rhenium analogue (Figure 8). The labeling yields of **8** at 10^{-5} M (>95%) and 10^{-6} (69, 52% at pH 5.0, 7.4) were significantly better than **7** as expected because of the smaller coordination ring.

Table 3. Labeling Yields (%) Observed for *fac*-^{99m}Tc$(OH_2)_3(CO)_3^+$ with the Respective Ligands at pH = 5.0 and 7.4

	pH = 5.0		pH = 7.4	
complex	10^{-5} M	10^{-6}	10^{-5}	10^{-6}
^{99m}Tc(N,S,N_{Py}-1)$(CO)_3^+$	>99	44	>99	86
^{99m}Tc(N,S,N_{Py}-4)$(CO)_3^+$	>99[1]	87[1]	>99[1]	87[1]
^{99m}Tc(O,S,N_{Py}-6)$(CO)_3$	92[1]	76[1]	90[1]	68[1]
^{99m}Tc(O,S,N_{Py}-7)$(CO)_3$	72	26	31	0
^{99m}Tc(O,S,N_{Py}-8)$(CO)_3$	>99	69	95	52

[1] Combined yield for the two species observed in the chromatogram.

Conclusions

A series of S-(pyridin-2-ylmethyl)-L-cysteine ligands were prepared and investigated with *fac*-M$(CO)_3^+$ (M = Re, ^{99m}Tc) to determine the preferred coordination mode, either tripodal (O,N,S) or linear (O,S,N_{Py}, N,S,N_{Py}). S-(pyridin-2-ylmethyl)-L-cysteine, **1**, was found to preferentially favor only the linear N,S,N_{Py} coordination mode among the three possibilities. The methyl ester protected version, **4**, helped elucidate the presence of N,S,N_{Py} diastereomers observed with **1** by limiting the coordination to a single mode. Independent structural characterization of the diastereomers (**5a**, **5b**) formed with **4** provided key insight into understanding the effect of ligand orientation on the metal center to the ^{1}H NMR spectra observed. The boc protected version, **6**, also provided indirect supporting evidence as the O,S,N_{Py} ligands were ineffective at complexing Re$(CO)_3^+$ under conditions examined. Only upon reducing the coordination ring size could a Re(O,S,N_{Py}-**8**)$(CO)_3$ product be observed in good yield.

^{99m}Tc$(CO)_3^+$ labeling studies with the S-(pyridin-2-ylmethyl)-L-cysteine ligands were effective at reasonable concentrations (10^{-5}−10^{-6} M) and correlated with the N,S,N_{Py} rhenium complexes, especially with the two diastereomer peaks observed with **4**. Surprisingly, ^{99m}Tc$(CO)_3^+$ complexed the O,S,N_{Py} ligands at 10^{-5} M illustrating a noteworthy difference between Re and ^{99m}Tc results, where further investigation with ^{99}Tc is needed. Overall, S-(pyridin-2-ylmethyl)-L-cysteine ligands have excellent potential with the N,S,N_{Py} coordination mode for M$(CO)_3^+$ (M = Re, ^{99m}Tc) applications with small molecules, peptides, and other targeting biomolecules.

Acknowledgment. The authors wish to thank Mary Dyszlewski of Tyco Inc. for the Isolink kits and

experimental protocols. The work was funded in part by the Department of Defense New Investigator Award Prostate Cancer (Grant W81XWH0510556), the Department of Energy Radiochemistry Education Award Program (Grant DEFG20705ID14-692IDNE006), and the Washington State University Start Up fund. Funding for the X-ray facility at UI was provided by the M. J. Murdock Charitable Trust and NSF-EPSCoR.

Supporting Information Available: Complete X-ray structural information for **2a**, **5a**, **5b**, and **9** is available as a CIF file, and ^{1}H NMR spectra of selected complexes as a PDF. This material is available free of charge via the Internet at http://pubs.acs.org.